ONTOGENETIC STUDIES ON SCENEDESMUS OBTUSIUSCULUS CHOD.

by

N. RAKOVÁN J. and MESZES G.

Department of Applied Botany and Histogenetics,

and Department of Plant Physiology of the Eötvös Loránd University, Budapest

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Introduction

In experiments on the ion uptake and enzymatic regulation in a synchronous culture of *Scenedesmus obtusiusculus*, by using culture techniques as described by Meszes – Komárek (1970), and an equipment as modified by Meszes – Sipos (1968), from time to time unusual values were obtained. The synchronous cultures were set up by Meszes and Sipos in such a way that the life cycle composed of asexual reproduction growth and asexual reproduction again could be repeated in every 24 hours and synchronized to a degree of 70 – 80%. In the synchronous cultures the material was adequately pretreated, and the temperature, as well as CO_2 and O_2 contents were constant. The light period took from 8 a.m. to 9. p.m. (13 hours) and the dark period from 9 p.m. to 8 a.m.

We have presumed that the different stages of the life cycle might influence the active ion uptake. The ontogenetic phases, may be well described in morphological terms by electron microscopic methods. The present paper reports the cytomorphologic changes *of Scenedesmus obtusiusculus* in the course of growth and asexual reproduction in order that the results can be compared with the former ion uptake studies (Meszes – Kralovánszky 1967).

Materials and Methods

The experiments were carried out with *Scenedesmus obtusiusculus* Chod. *(Chlorophyceae)* No 5618 obtained from the alga collection of the Biological Research Institute, Hungarian Academy of Sciences, Tihany. For electron microscopic observations samples were taken from the synchronous culture every 1 – 2 hours, fixed in 1% $KMnO_4$, dehydrated in alcohol and embedded in durcupan. Control samples were taken from a

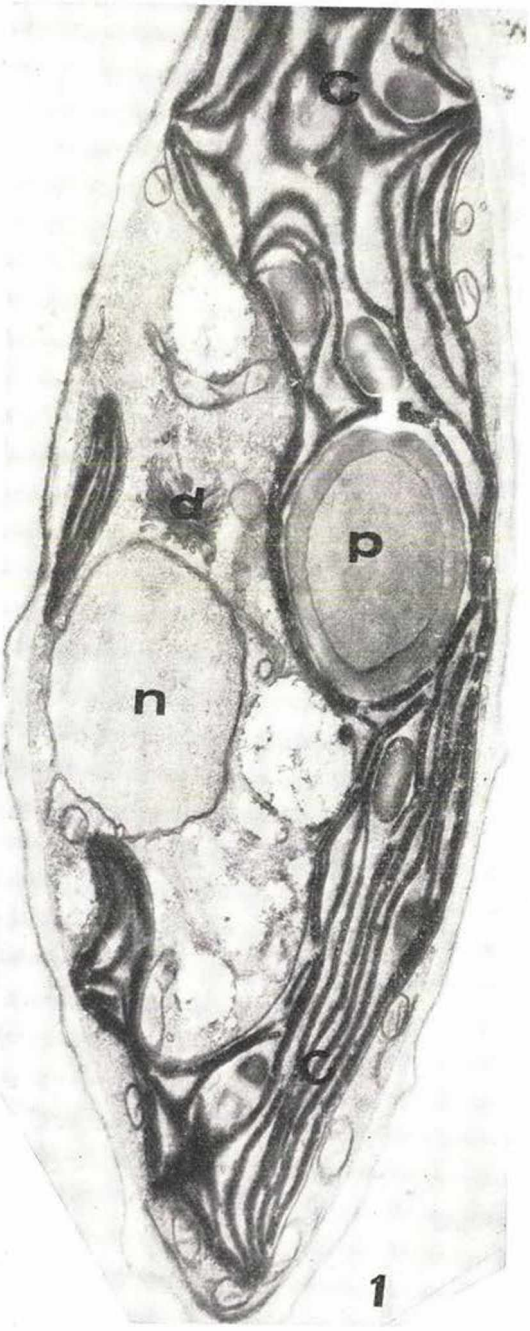

Fig. 1. *Scenedesmus obtusiusculus* sectioned parallel to the long axis. c = chromatophore, p = pyrenoid, n = nucleus, d = dictosome 16,500x

Fig. 2. *Scendesmus obtusisculus* sectioned perpendicular to the long axis. c = bell-shaped chromatophore 13,500x

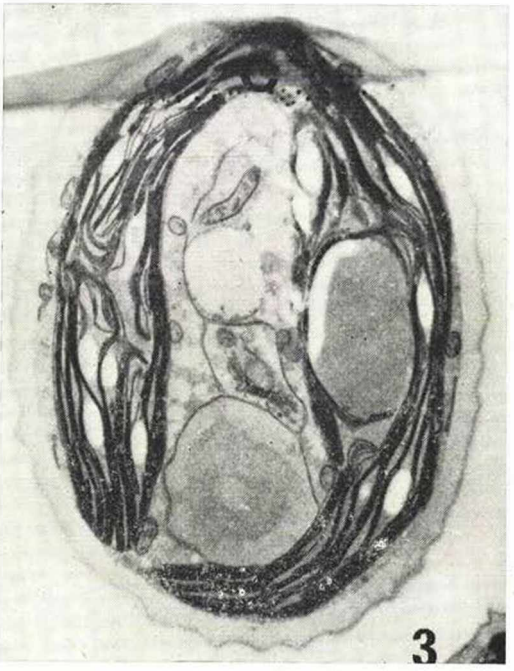

Fig. 3. Diving chloroplast 11,000x

non-synchronous culture. Sections were prepared with a Porter-Blum ultramicrotome, stained with uranylacetate and lead citrate. An electron microscope type KEM I. was used.

Results

Our experimental material consisted of cell populations of the unicellular green alga *Scenedesmus obtusiusculus*. The members of the cell populations differed from each other in shape and size even in the light microscope. These differences were more remarkable in the electron micrographs. However, even so some general conclusions can be drawn.

Generally, *Scenedesmus obtusiusculus* is a longish, spindle-shaped, non-thorny organism, 6 to 12 μ long and 2,5 to 6 μ wide depending on the stage of development. Sectioned parallel to the long axis the nucleus, the chloroplast with pyrenoid and starch granules, and one of the two dictyosomes and mithochondria can be seen (Fig. 1). Sectioned at right angle to the long axis the single bell-shaped chloroplast and nucleus can be recognized. The nucleus is in the gap of the chloroplast in the dorsal part of the cell. Chloroplast contains grana (Fig. 2). The ultrastructure

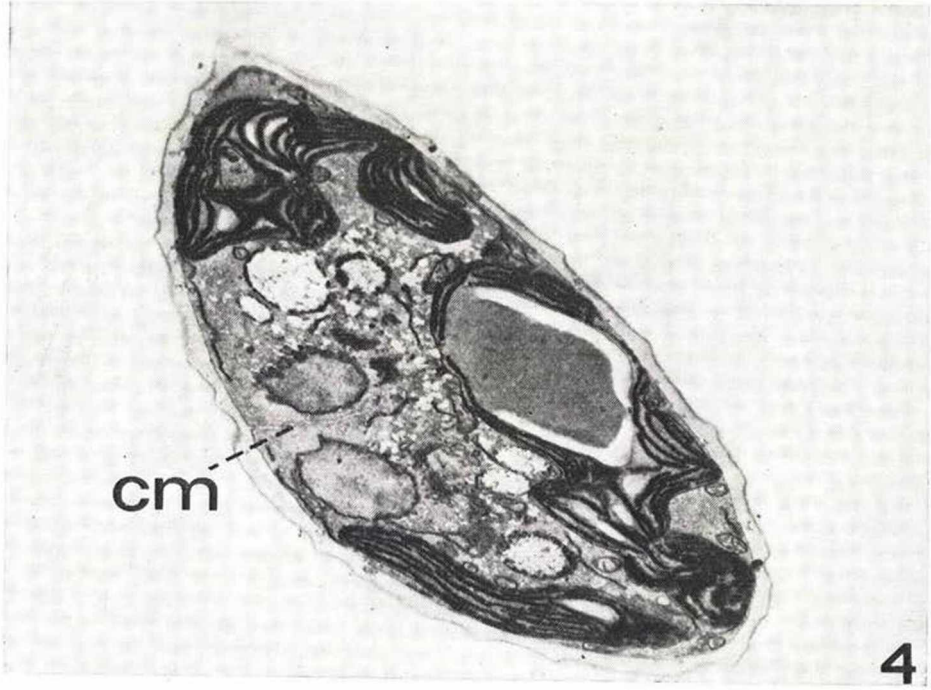

Fig. 4. Nuclear division, duplication of dictyosome and folding up of the cell membrane. cm = cell membrane 11,000 x

of chloroplast membranes of *Scenedesmus* was studied by W e i e r et al (1966).

Even in natural alga populations the life processes follow a more or less daily rhythm, for the photosynthesis requires light and the growth and mainly the reproduction generally take place in the dark. In synchronous cultures this rhythm is uniform (synchronized).

In addition, *the intensity* of division is also influenced or the time of division brought forward in synchronous cultures. For instance, the chromatophore division begins still in the light period at about 5 p.m. This division is perpendicular to the chromatophore membranes and takes place always opposite to the nucleus (Fig. 3). Rarely, dividing chloroplasts occur as early as 11 a.m.

Together with the chloroplast division or following it, mitochondria also undergo changes. Most probably they fuse for they reach a many times greater size than the normal ones (Fig. 8).

Shortly after the duplication of the chloroplast, nuclear division follows. This seems to reach its highest intensity at 7 p.m. (still in the light period). It is remarkable that in the course of the nuclear division the dictyosomes also duplicate themselves, similarly to Botrydium granula-

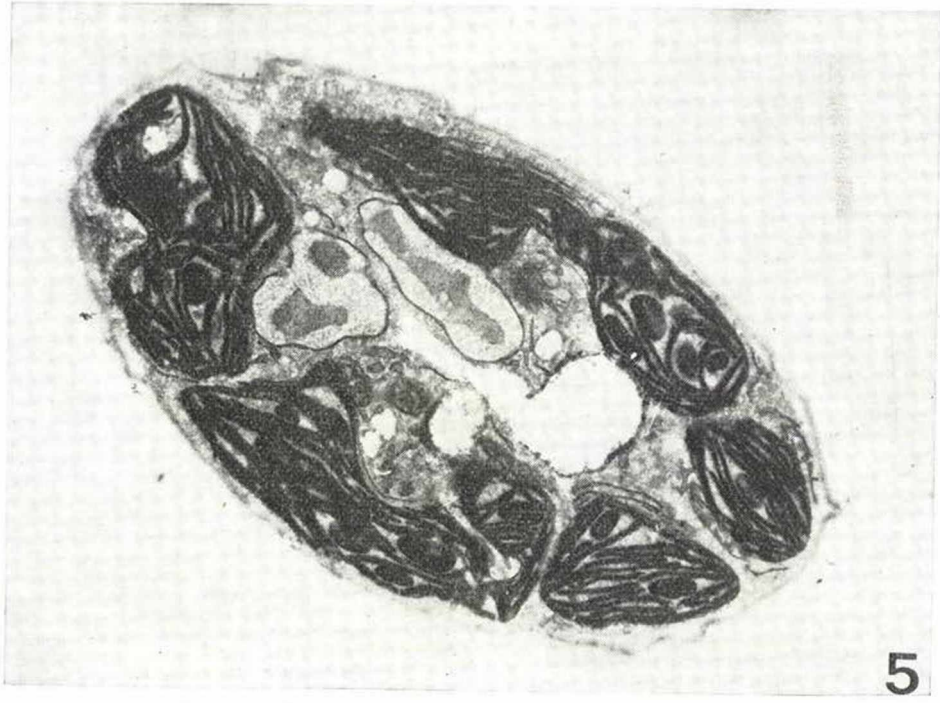

Fig. 5. Division of chromatophores, appearance of autospores. 11,000x

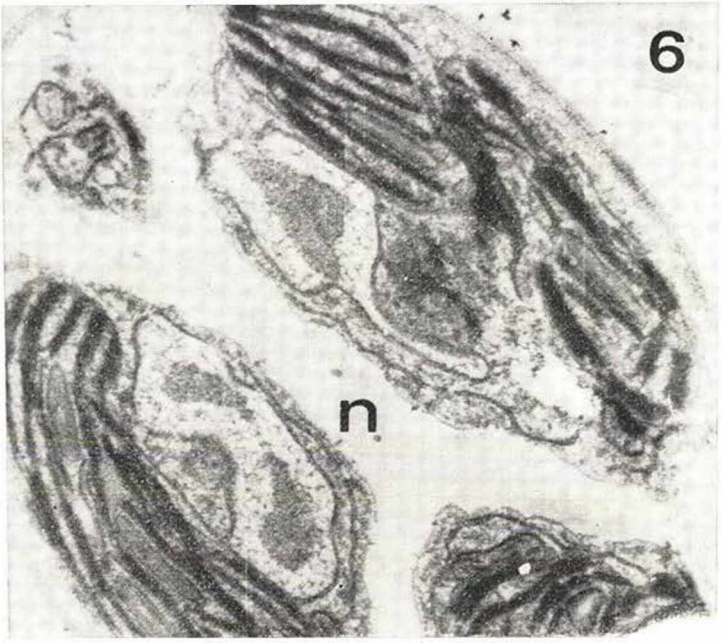

Fig. 6. Nuclear division as amitosis. n = nucleus 22,0000x

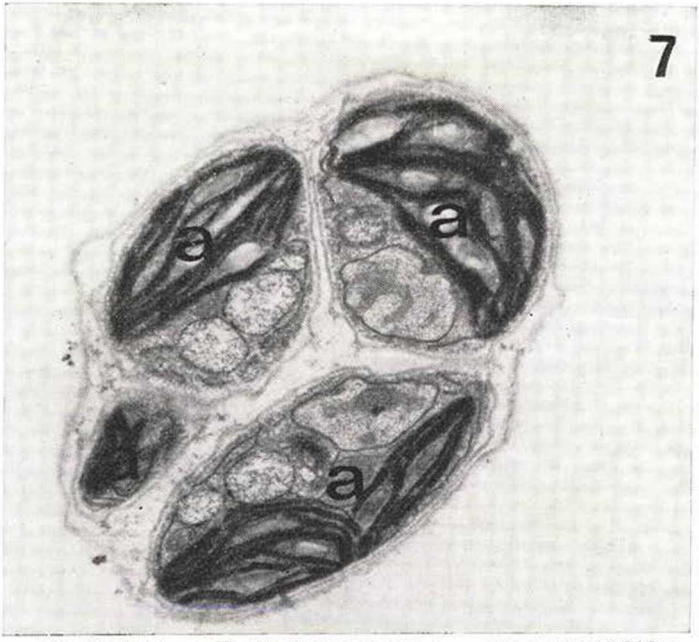

Fig. 7. Mother cell with 4 autospores. a = autospore 13,000x

tum (Nagy – Fridvalszky 1968; N. Rakován – Fridvalszky (1970). Simultaneously with the nuclear division – next th the nucleus – the cell membrane folds up digitiformly. The first cellular division is perpendicular to the long axis (Fig. 4). At 7 p.m. the chloroplast divides again, in such a way that the site and shape of the future daughter cells appear (Fig. 5).

Scenedesmus obtusiusculus produces mostly 4 autospores but 3 or 6 – 8 autospores can also proceed from it (Fig. 7, 9, 10, 11).

The second and third nuclear divisions mostly appear at 8 p.m. and they may be amitotic (Fig. 6). The cell wall of the daughter cells begins to thicken likewise at 8 p.m. and this process continues for 1 to 2 hours. At the same time the wall of the mother cell slowly resolves and between midnight and 2 a.m. the autospores leave the mother cell. In this way, one developmental cycle is finished or rather the other cycle begins (Fig. 11). The chromatophores reach their significant bell-shaped character long after the liberation of the autospores.

Cells taken from non-synchronous cultures are rather thick-set and als the cell walls are thicker than the synchronous ones.

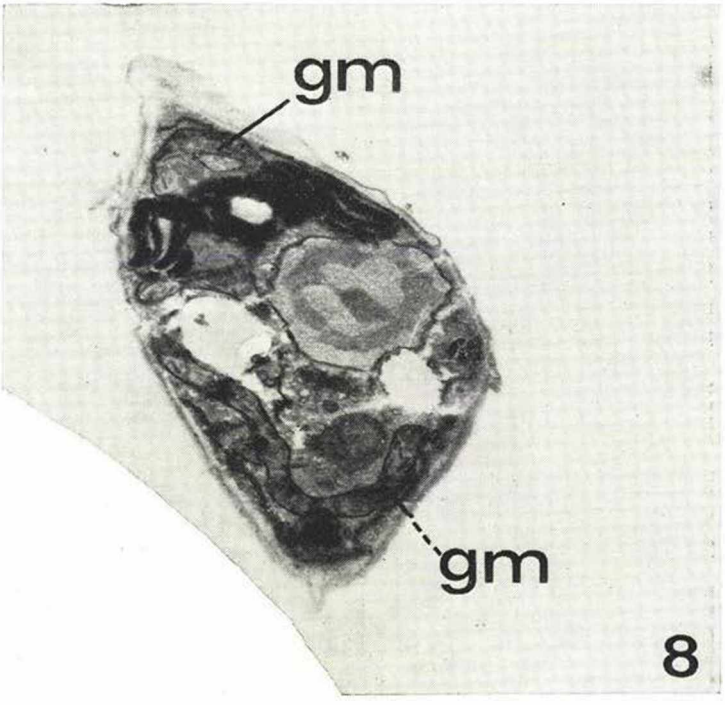

Fig. 8. Fusion of mitochondria. gm = giant mitochondrium 13.000x

Discussion

In synchronous cultures mostly two green alga genera *Chlorella* and *Scenedesmus* are cultivated. Their ultrastructural changes in connection with the life cycle were studied by some workers. However, the relationship between the changes in the fine structure during ontogenesis and the changes in ion uptake has not been elucidated.

In *Chlorella ellipsoidea* cultures of 48 hours the life cycle was observed under EM by M u r a k a m i et al (1963). Essentially our results agree with theirs although in contrast with our findings were able to investigate the liberation of autospores from the mother cell already in the light phases.

K o m a r e k (1968) has studied the synchronous culture of Scenedesmus consisting of 4 cell coenobia. Using light microscope he has found that the autospores firstly begin to develop in the two internal cells and only following that in the two marginal cells of the coenobium. A similar formation was observed in the case of a 8-cell coenobium. It is difficult to use data from such culutres for finding connections between the phases of the life cycle and the rate of active ion uptake.

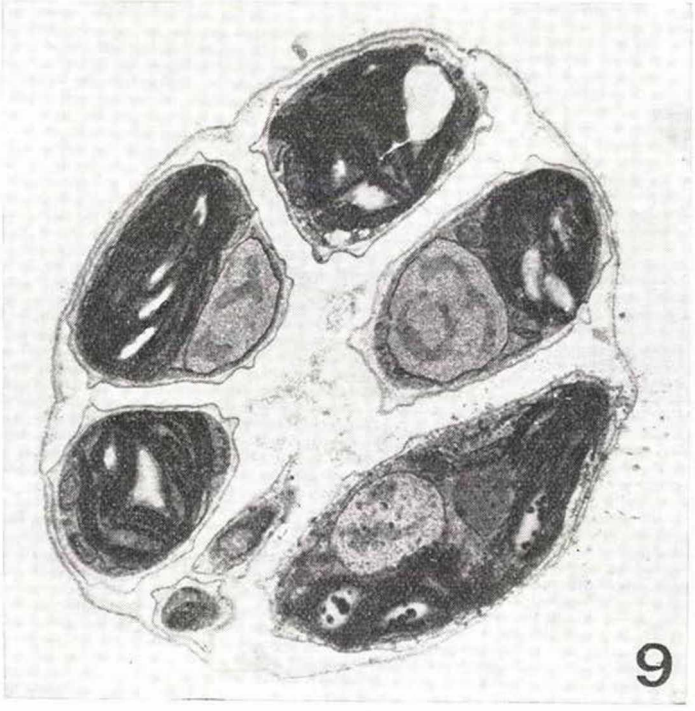

Fig. 9. Mother cell with 6 (7) autospores. 13,000x

In this study our intention was to describe cytomorphological some ontogenetic phases of *Scenedesmus obtusiusculus* in synchronous conditions and to point out the approximative times and the developmental stages when the changes could influence the rate of ion uptake in the course of ontogenesis.

The changes in the course of the observed period of the life cycle are in close correlation to biochemical processes. This correlation is proved by the fact that when the division of the cellular organelles takes place, ion uptake and ATPase activity increase simultaneously. On the other hand, during the formation of the daughter cell, the thickening of the new cell walls, and the liberation of the autospores these activities are known to be markedly reduced. (Meszes – Kralovánszky et al. 1967; Meszes – Erdei 1969a; 1969b.)

In cytomorphologic relation the following conclusions can be drawn: a) we could demonstrate the dictyosome division together with the nuclear division in the case of Scenedesmus, b) the second and third nuclear divisions may take place as amitosis. It is possible that the observed expansion of the mitochondrium has an influence on the intensity of respiration i.e. indirectly on the rate of activ ion uptake.

Thanks are due to Mrs. K. Petrovits technician for her help.

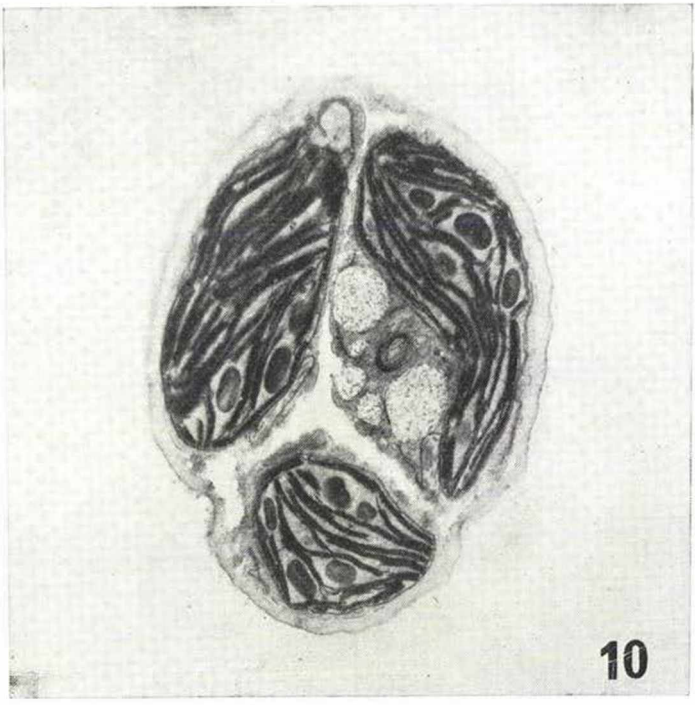

Fig. 10. Mother cell with 3 autospores. 13,000x

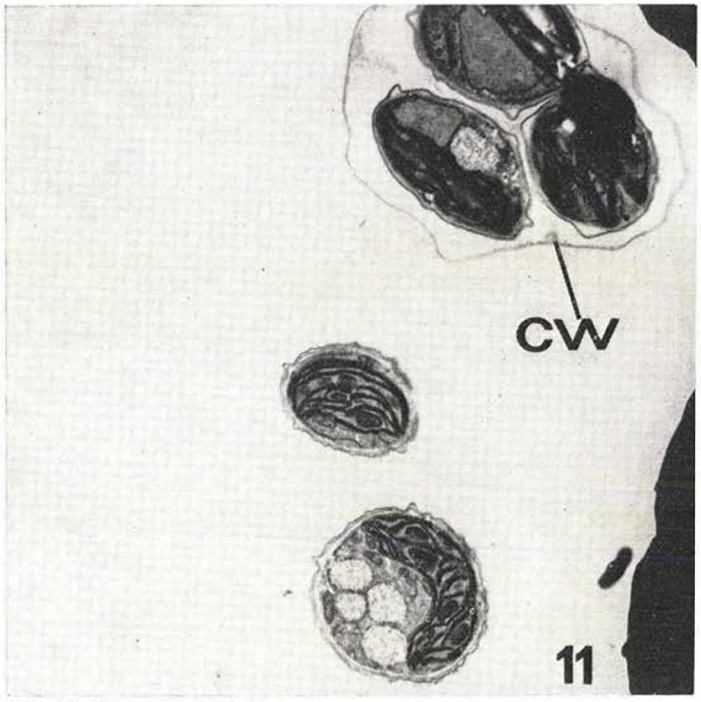

Fig. 11. The cell wall of the mother cell resolves and the new alga cells liberate. cw = cellwall. 4,400x

Summary

We have studied the life cycle of *Scenedesmus obtusiusculus* Chod. in synchronous culture and determined the phases and approximate time duration of development by electron microscope. The developmental phases studied may have an influence on active ion uptake.

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